THE MODIFICATION OF THE ACTION OF INSULIN BY PITUITARY EXTRACT AND OTHER SUBSTANCES.

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THAT the pituitary body has some undoubted connection, direct or indirect, with the ordinary processes of metabolism was made clear by the observations of Cushing(1) and his collaborators, who found that in cases in which there existed in the organism a hypophyseal deficiency, whether this occurred clinically or was produced experimentally, there was "an acquired high tolerance for sugars, with the resultant accumulation of fat," whilst the blood became hypoglycæmic. Apart, however, from this extremely suggestive point, the information available has been of a meagre and puzzling nature. Much time has been given to the determination of the effect of infundibular extract on the normal blood sugar. The assertion of Borchardt(2) that this extract caused the sugar in the blood to rise, was in turn denied by Franchini(3) and reaffirmed by Cushing(1), who showed that intravenous administration was "almost without exception" followed by hyperglycæmia. No progress, however, was made in comprehending the mechanism of this rise of the blood sugar, or in relating it in any way with that produced by adrenaline.

The problem was complicated, rather than simplified when Stenström(4), working in Bang's laboratory, showed that doses of infundibular extract were able to depress or inhibit the adrenaline hyperglycæmia and glycosuria in rabbits. This observation was confirmed by my own experiments(5), which suggested the desirability of investigating the effect of pituitary extract on the hypoglycæmia produced by insulin. From its antagonism to adrenaline hyperglycæmia it might have been expected that it would reinforce the effect of insulin in lowering the blood sugar. As a fact it was found that the pituitary extract had a powerful antagonistic effect on the action of insulin also. Experiments which demonstrate this antagonism are here described, together with others which have a bearing on the meaning of the changes in blood sugar produced by pituitary extract and adrenaline.

Methods. The experiments were made on unanæsthetised rabbits, and samples of blood were obtained from the ear vein. Determinations

of the level of the blood sugar were in all cases made on 1 c.c. blood according to the method described by Shaffer and Hartmann(6). The samples of insulin used were two which were prepared by Dr H. W. Dudley, to whom my thanks are due. The preparations of pituitary extract were obtained partly from the posterior lobes of fresh ox pituitaries, dissected free from pars intermedia and anterior lobe, and extracted with water according to a routine recently described(7), and partly from some dried posterior lobe material, similarly extracted; one or two experiments were performed, in addition, with the commercial extract "Infundin," made by Burroughs, Wellcome and Co.

The effect of infundibular extract on the fall produced by insulin. For the purpose of the experiments there were available nine rabbits, whose reactions to various doses of one sample of insulin had been carefully recorded. While the fall in the percentage of blood sugar produced by a given dose varied enormously in different rabbits, it was fairly regular for any one animal, and in two rabbits, nos. 7 and 9, it had shown, in four consecutive experiments, a striking uniformity.

The nature of the action to be described may be seen from the details set out in Table I and Fig. 1. On three occasions, rabbit 7 was kept

TABLE I (see Fig. 1).

Rabbit 7. Wt. 3·2 kgm. No food since previous day in each case.

	Injection (subcutaneous)		
Blood sugar %	Insulin B 4·7 mgm.	Insulin B 4·7 mgm. + 4 c.c. fresh infundibular extract (2·5 %)	4 c.c. fresh infundibular extract
Before injection	·112	·122	·125
40 mins. later	·062	·118	·154
80 ,,	·043	·106	·122
120 ,,	·038	·118	·107
175 ,,	·041	·108	·099
216 ,,	·051	—	·098

Rabbit 9. Wt. 3·8 kgm. No food since previous day in each case.

	Injection (subcutaneous)		
Blood sugar %	Insulin A 11·4 mgm.	Insulin A 11·4 mgm. + 4 c.c. B.W. "Infundin"	4 c.c. B.W. "Infundin"
Before injection	·106	·116	·117
40 mins. later	·061	·128	·118
80 ,,	·053	·101	·096
120 ,,	·049	·085	·116
180 ,,	·046	·086	·121
240 ,,	·068	·107	·131

without food, though given water, for a period of 18–20 hours. At the end of this time, on the first occasion, a subcutaneous injection of

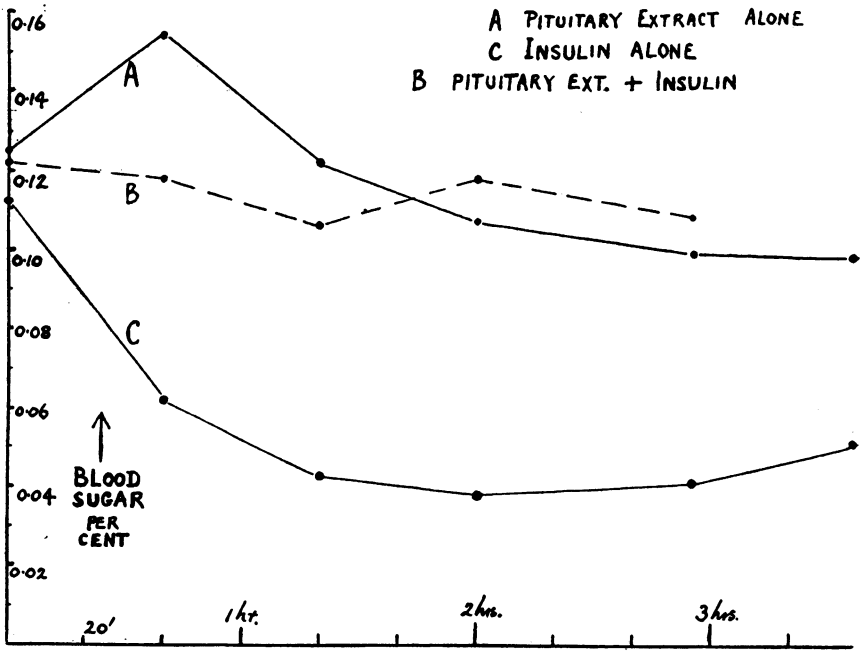

Fig. 1. For description see text.

4·7 mgm. of a batch of insulin was given. Samples of blood were withdrawn from the ear vein before the injection, and at various intervals after, and these showed that, in 2 hours from the injection, the percentage of blood sugar had fallen from ·112 to ·038. On the second occasion the same dose of insulin was injected into the loose skin of one flank, and immediately afterwards 4 c.c. of a 2·5 p.c. extract of fresh infundibular material were similarly injected into the opposite flank. At the end of 2 hours the percentage of sugar in the blood had not fallen at all, but, within the error of the method, was at the same level as before the injections took place. On the third occasion a similar dose of infundibular extract to that previously used was injected by itself, in order to demonstrate its effect on the blood sugar. After a relatively small initial rise, obvious at the end of 40 minutes, the blood sugar actually fell, being at the original level in 80 minutes, and clearly below it in 2 hours; the fall was maintained during the third and fourth hours.

The results obtained with rabbit 9, using a different preparation of

insulin and a commercial sample of posterior lobe extract, were in every way similar to those described for rabbit 7. Such results were confirmed on several other rabbits and demonstrated that injections of infundibular extract were able, according to the dose given, either to diminish or to abolish the fall produced by an injection of insulin.

The cases shown in Table I made it additionally clear that this effect of infundibular extract was not to be explained by its power when given alone of producing an increase of the blood sugar which, by algebraic summation, could compensate for the fall produced by insulin.

With reference to the effect of pituitary extract on the normal blood sugar, the behaviour of rabbit 7 was of some interest. The effect of subcutaneous injection, if any, is usually stated to be a rise. In the three experiments of this kind on rabbit 7, there was, in each case, a transient rise of blood sugar, followed in 2 hours by a definite though slight fall, lasting at least 2 hours longer. In two other rabbits, again, the *only* effect of subcutaneous injection of pituitary extract was a slight fall. In five other experiments, there occurred a simple rise of blood sugar of brief duration, and finally, in two experiments, the rise was considerable and prolonged. While, therefore, these experiments confirmed the view that in some cases pituitary extract injected subcutaneously does cause hyperglycæmia, they also showed that, in others, the opposite condition may result, and that, even in these, the simultaneous injection of pituitary extract prevents the fall of blood sugar caused by insulin.

The specific nature of the effect. Extracts of spleen, thymus, brain tissue, desiccated thyroid material, and anterior lobe of the pituitary gland were prepared and tested. With the exception of those from the thyroid and the anterior lobe, the extracts were obtained from the fresh tissues of the rabbit; the extract of thyroid was made from desiccated sheep's thyroid, and the anterior lobe extract from fresh ox pituitaries. The extraction was in all cases identical with that adopted for infundibular material, namely an extraction of weighed minced tissue, with a known amount of water, which was then faintly acidulated, boiled and filtered. The results with these extracts were uniformly negative; none of them affected, in either direction, the action of insulin, as determined in a control experiment on the same animal with the same dose of the same batch of insulin.

Since it is known that histamine occurs in many organ extracts, an experiment in which 3 mgm. histamine di-phosphate was injected simultaneously with a small dose of insulin was carried out. Again the

result in no way differed from that of the control experiment without histamine.

A final proof of the specificity of the action of infundibular extract was obtained by taking advantage of the fact (Guggenheim(8), Dudley(9)) that the oxytoxic and pressor principles present in this extract are destroyed by exposure for 2 hours to the action of normal sodium hydroxide at room temperature. A sample of pituitary extract treated in this way and subsequently neutralised, was found to have lost not only its activity on the isolated guinea pig's uterus, but also the power to prevent the fall of blood sugar produced by insulin. The following experiment illustrates this:

To a volume of posterior lobe extract, an equal volume of 2*N* NaOH was added. The mixture was allowed to stand for 1½ hours at 37° C. and then neutralised. A volume of this solution, representing a dose of the original extract known to abolish the fall produced by a given dose of insulin, was then used for injection. The control experiments are shown:

Rabbit 10. Wt. 3·7 kgm.

	Injections		
Blood sugar %	7·4 mgm. Insulin A alone	7·4 mgm. Insulin A + 3 c.c. 1 % ext. of dried infundibular material	7·4 mgm. Insulin A + same dose of inactivated infundibular extract
Before injection	·123	·169	·138
40 mins. after	·085	·196	·084
60 „	·080	·188	·082
80 „	·086	·174	·079
100 „	·087	·171	·085
160 „	—	·139	·084

The third column of figures shows that after treatment of the pituitary extract with soda, its effect on the fall of blood sugar due to the insulin had disappeared. Hence it was evident that the ability of pituitary extract to suppress the effect of insulin depended on its content of a substance having the same instability to alkali as the known active principles.

The effect of pituitary extract on hypoglycæmia. As Banting, Macleod and their colleagues(10) have shown, a rabbit, after a large dose of insulin, passes into a condition in which periods of collapse alternate with periods of violent convulsions. It was found that, provided the dose was adequate, subcutaneous injection of infundibular extract never failed to remove all these symptoms in not more than 10 minutes from the time of injection, the recovery closely resembling that observed after the injection of dextrose. The following experiments, in which relatively

large doses of posterior lobe extract were used, show that the disappearance of convulsions was in fact attended by a rapid elevation of the previously low percentage of sugar in the blood.

1. Rabbit. Wt. 2·6 kgm. (see Fig. 2).

Injection	Time	Symptoms	Blood sugar
20 mgm. insulin ...	Zero	—	·134
	1 hour	—	·051
	1½ hours	Temporary convulsions	—
	2 „	—	·048
	From 2 h. 40 m. to 2 h. 57 m.	Continuous convulsions and collapse	—
5 c.c. 10 % commercial infundibular extract	2 h. 57 m.	—	—
	3 h. 7 m.	Normal	—
	3 h. 20 m.	—	·092

2. Rabbit. Wt. 2·6 kgm.

Before injection of insulin ...	Blood sugar ·109 p.c.	
After injection 1 hour	... „	·047
„ 2 hours	... „	·045
„ 3 „	... „	·035
„ 3 h. 5 m.	... Convulsions	
„ 3 15	... 4 c.c. 2 p.c. extract dried infundibular material injected subcutaneously	
„ 3 20	... Convulsions	
„ 3 25	... Rabbit sits up and appears normal	
„ 3 35	... Blood sugar ·065 p.c.	

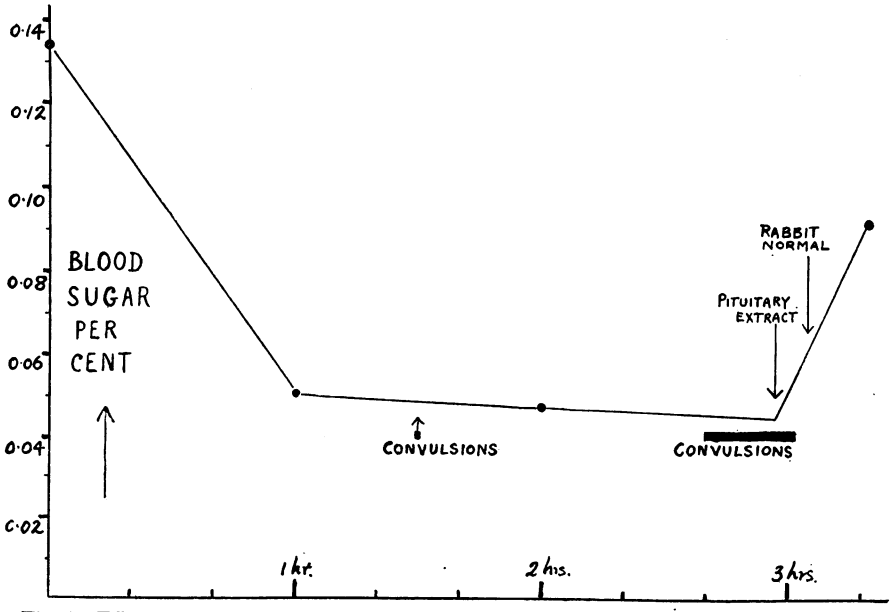

Fig. 2. Effect of pituitary extract on the blood sugar of an animal in hypoglycæmic convulsions. Insulin injected at zero.

In the first of these experiments a rabbit received an injection of insulin great enough to produce, after 160 minutes, a series of convulsive fits, in the intervals between which the animal was seen to be in a condition of collapse. At this point the percentage of blood sugar was certainly not greater than ·048 p.c., a value observed one hour previously, and probably was below ·04 p.c. Subcutaneous injection of pituitary extract led to the complete recovery of the animal within 10 minutes, and a sample of blood withdrawn 23 minutes after the injection contained ·092 p.c. sugar.

The effect of adrenaline on insulin hypoglycæmia. Since it has already been shown by Banting, Best, Collip, Macleod and Noble(11) that there exists an antagonism between the effect on the blood sugar of adrenaline and that of insulin, some experiments were carried out to see what points of difference there were between the action of adrenaline and of pituitary extract in this respect. The observations on the relation of adrenaline and insulin fully confirmed what was found by the Toronto workers, for it was clear that the hyperglycaemic effect of a given dose of adrenaline was greatly reduced when the dose was given to an animal already under the influence of insulin. The experiments

TABLE II. Rabbit 1. Wt. 3 kgm. On occasion of each experiment, it was kept without food for 18–20 hours previously.

Exp.	Time	Blood sugar %	Injection (subcut.)
A	Just before injection	·093	—
	Zero	—	12 mgm. insulin B
	100 m. later	·044	—
	170 ,,	·034	—
	220 ,,	·031	—
	280 ,,	·052	—
B	Just before injection	·110	—
	Zero	—	·5 mgm. adrenaline
	38 m. later	·169	—
	63 ,,	·233	—
	90 ,,	·291	—
	128 ,,	·337	—
C	Before injection	·120	—
	Zero	—	4 c.c. "Infundin"
	20 m. later	·152	—
	50 ,,	·157	—
	90 ,,	·113	—
	120 ,,	·124	—
D	Just before injection	·123	—
	Zero	—	12 mgm. insulin B
	158 m. later	·050	—
	166 ,,	—	0·5 mgm. adrenaline
	186 ,,	·051	—
	211 ,,	·069	—
	255 ,,	·077	—
E	Before injection	·121	—
	Zero	—	12 mgm. insulin
	40 m. later	·064	—
	80 ,,	·053	—
	100 ,,	·041	—
	158 ,,	·036	—
	160 ,,	—	4 c.c. "Infundin"
	182 ,,	·084	—
	210 ,,	·071	—
	254 ,,	·066	—

described in Table II were all carried out on one rabbit. They show the effects of insulin, pituitary extract and adrenaline when given singly, as well as the antagonism of the two last for insulin. It will be seen that the effect of the chosen dose of adrenaline alone in producing hyperglycæmia is not only much greater (·110–·337 p.c.) than that of the dose of pituitary extract (·120–·157) but much more prolonged; on the other hand the antagonistic effect of the same dose of pituitary extract on insulin hypoglycæmia is more extensive, more persistent and much more rapid in onset than that of adrenaline.

The effect of pituitary extract on adrenaline hyperglycaemia. In view of these effects, it seems relevant to give an example (Table III) showing the relation between infundibular extract and adrenaline on the blood sugar of a normal animal. As already stated, Stenström(4) originally described the inhibition of adrenaline hyperglycaemia and the suppression of adrenaline glycosuria in rabbits by infundibular extract. These findings were confirmed by me in experiments, of which the details were not published.

TABLE III. Rabbit. 2·8 kgm. In each case food was withheld for 18–20 hours before the experiment.

	Blood sugar percentages	
Time	After injection of ·5 mgm. adrenaline	After injection of 5 mgm. adrenaline + 4 c.c. pit. ext. "Infundin"
Before injection	·107	·111
40 m. after	·195	·127
80 ,,	·277	·123
120 ,,	·329	·144
180 ,,	·325	·198
240 ,,	·269	·252

The changes observed are characteristic of those seen in these circumstances; some evidence of the adrenaline action is usually visible in spite of the injection of infundibular extract. Nevertheless at the end of 2 hours from the injection, Table III shows that a rise due to adrenaline alone amounting to ·22 p.c. has been reduced, under this inhibiting influence to one of only ·03 p.c. A similar effect is observed on the hyperglycæmia produced in rabbits by ether anæsthesia provided that the dose of pituitary extract is large, and is administered before the induction of the anæsthetic.

The effect of ergotoxine on adrenaline hyperglycæmia and on insulin hypoglycæmia. There are two additional experimental results which have significance for an analysis of the relation of the factors which influence

the circulating glucose. The first of these was originally described by Miculicich(12) and confirmed by myself; it was that the glycosuria and hyperglycæmia produced by adrenaline are inhibited by an intravenous injection of ergotoxine. The suppression is more nearly complete than that produced by pituitary extract. It was accordingly of interest to examine the effect of ergotoxine on insulin hypoglycæmia. Instead of ergotoxine, ergotamine tartrate was used, which has recently been shown by Dale and Spiro(13) to be identical, quantitatively and qualitatively, in its physiological properties with ergotoxine. It was found that following an intravenous injection of ergotamine, the amount of insulin adequate to produce convulsions was much less than in the normal animal. Fig. 3 shows the course of the changes in the blood sugar in two experiments on a well fed rabbit. In one experiment (A) 8 mg. of insulin was given alone. In the other (B) 5 mgm. of ergotamine tartrate was first given and 2 hours later 8 mgm. of the same sample of insulin.

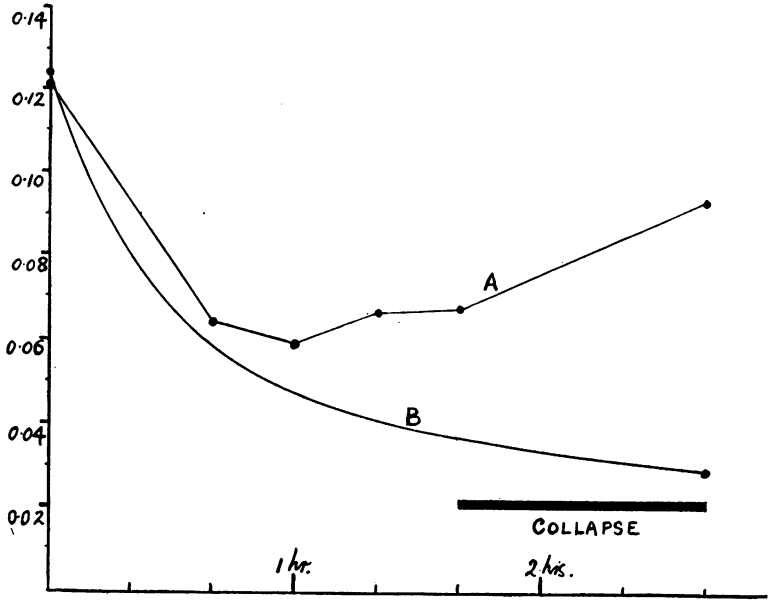

Fig. 3. Curve A shows the effect of a small dose of insulin on the blood sugar of a well-fed rabbit. Curve B shows the effect of the same dose given 2 hours after an intravenous injection of 5·0 mgm. ergotamine tartrate in the same animal.

It will be seen that the injection of insulin alone produced a transitory fall, 1 hour later, to the neighbourhood of ·060 p.c., and the last traces of its effect were disappearing in 2 hours 40 minutes, and that following

the ergotamine administration, the sugar, under the influence of the same amount of insulin, fell to ·029 p.c.; the animal passed from convulsions to a condition of complete collapse, so that blood was only obtained for analysis by cardiac puncture; the animal would certainly have died but for an intravenous injection of dextrose.

Discussion.

While it is a matter of some difficulty to fit all of the preceding results into a consistent scheme, there is at least one action the mechanism of which seems reasonably certain. It is generally agreed that the hyperglycæmia produced by adrenaline is due to conversion of liver glycogen into glucose, and the fact that this, like other "motor" effects of adrenaline, is paralysed by ergotoxine, shows that it is due to action on a mechanism in the liver associated with sympathetic innervation; in other words, the adrenaline hyperglycæmia is simply one of its sympathomimetic actions.

The recent work of Macleod and his colleagues(14) suggests that while the initial fall of blood sugar caused by insulin is independent of the amount of liver glycogen, the rate at which the blood sugar returns to its normal level, as the effect of the insulin passes off, is largely determined by the amount of sugar which the liver can supply. This view is fully confirmed by the observation that ergotoxine greatly increases the action of insulin; it may be supposed that the alkaloid, by paralysing the sympathetic supply to the liver, prevents this organ from carrying out its compensatory function, so that a small dose of insulin has not to deal with a continuous supply of sugar from the liver, and is therefore able to produce an almost complete disappearance of circulating glucose.

This view further explains the observations here recorded with adrenaline. The experiments described show that the hyperglycæmic action of a given dose of adrenaline in the normal animal is reduced to one-sixth of its value in the same animal 2 hours after the injection of insulin. If in this condition the liver is already producing sugar as rapidly as possible, the additional stimulus of adrenaline will be proportionately less effective.

There remains the relation of pituitary extract to carbohydrate metabolism. Cushing's work suggests that the specific antagonism, between the active principle of the pituitary posterior lobe and insulin, here described, is a factor of importance in normal metabolism. Cushing found that the patient suffering from hypopituitarism, or the animal

deprived of its pituitary body, developed a condition which was the antithesis of that of the diabetic. There was an enormously increased sugar tolerance which led to adiposity, and frequently there was hypoglycæmia. We have now evidence that pituitary injections inhibit the action of insulin in producing hypoglycæmia, and that, when given to an animal in hypoglycæmic convulsions, they produce a rapid recovery, due to return of the blood sugar towards the normal. These facts suggest that carbohydrate metabolism is influenced by pituitary extract in a precisely opposite sense to that in which it is affected by insulin.

It is not yet clear to what the hypoglycæmia produced by insulin is due. On the one hand there is the evidence of Dixon and Pember, quoted by Macleod and others(14) of a raised respiratory quotient following the injection of insulin, and on the other hand the evidence of Dudley, Laidlaw, Trevan and Boock(15), who have described a diminished respiratory exchange, suggesting that the sugar is dealt with in some other way than by combustion. If insulin were to promote increased combustion of sugar, we should conclude that pituitary extract prevented this taking place; but then, and indeed on any available view of the mechanism of the action of insulin, it is difficult to explain the origin of the sugar which is almost immediately liberated in the system of a hypoglycæmic rabbit by an injection of pituitary extract. It is extremely hard to believe that the source of this is the liver. We know that pituitary extract prevents, in rabbits, not only the glycosuria and hyperglycæmia due to adrenaline, but also that due to anæsthetics, in both of which cases pituitary extract must have a deterrent effect on glycogenolysis. It does not appear credible that pituitary extract should prevent adrenaline hyperglycæmia by suppressing the conversion of glycogen into sugar, and also prevent insulin hypoglycæmia by accelerating the same process[1]. Further discussion of the exact meaning of the antagonism between pituitary extract and insulin must obviously be postponed until the mode of action of insulin itself is more completely understood. The point here to be emphasised is that while adrenaline apparently opposes the action of insulin simply in virtue of its accelerating effect on the normal compensatory action of the liver, the antagonism of pituitary extract for insulin is of a more direct and specific nature, and is not shared by extracts of the other tissues which have been examined.

[1] Direct evidence against the view that insulin hypoglycæmia is due to glycogen formation is shortly to be published by Dr H. W. Dudley.

SUMMARY.

1. Subcutaneous injections of extract of posterior lobe of the pituitary gland given simultaneously with injections of insulin, diminish or abolish the fall of blood sugar produced by the latter. The doses of pituitary extract used do not, when given alone, produce a rise of blood sugar sufficient to explain this inhibition of the action of insulin as being the result of an algebraic sum.

2. This effect is not produced by similar extracts of anterior lobe of pituitary, or of spleen, thyroid, brain tissue or thymus. Nor is it produced by histamine. The property, in the case of posterior lobe extract, is destroyed by treatment with *N*-alkali in the cold.

3. Pituitary extract removes the symptoms of hypoglycæmic convulsions, causing a rapid elevation of the blood sugar.

4. The effect of a small dose of insulin is greatly increased by previous intravenous injection of ergotoxine.

5. Confirmation is given of the following points: (*a*) pituitary extract inhibits adrenaline hyperglycæmia and glycosuria; (*b*) ergotoxine abolishes adrenaline hyperglycæmia and glycosuria; (*c*) insulin greatly reduces the hyperglycæmic action of adrenaline.

6. Cases are given in which subcutaneous injections of pituitary extract in the normal animal led to a fall of blood sugar, though as a rule such injections cause a transient rise.

My thanks are due to Dr H. W. Dudley for the samples of insulin used, and to Mr H. P. Marks for carrying out some of the estimations.

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